Phenomics for Abiotic Stress Tolerance - Challenges Ahead

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1. Introduction

In the next fifty years, planet earth is going to have a burden of 8-10 billion of human population, 25-50% more degraded land, additional 48.6% CO_2 in atmosphere and a temperature increase by at least 2°C (Jaggard et al., 2010). The impact of such dramatic changes on plant ecology and crop cultivation system projects a frightening scenario in the next fifty to hundred years. A United Nations report has estimated that the climatic changes together can cause a fall in food production by 25%, resulting in more hunger, increased price of food and increasing possibilities of war over food. With a current rate of 10 ha minute^{-1} soil degradation and 23 ha minute^{-1} deforestation at global level as stated in UNCCD Secretariat Recommendations for Policymakers published in April, 2012, increasing food production is going to be indeed a herculean task and attaining food security in the next 100 years possibly seems unachievable. The greatest hindrance for increasing crop productivity will invariably come from the various abiotic stresses including drought and desertification from water scarcity (both for irrigation and groundwater) and deforestation, anoxia from waterlogging, toxicity caused by water unsuitable for crop cultivation and salinity with poor nutrient availability in the degraded lands. Developing crop genotypes performing well under such complex environment conditions is going to be much more difficult that what we have achieved in the past era of high yielding varieties which perform better under optimal and favourable conditions.

The crop researchers thus are faced with new, baffling challenges which need to be combated with more speed, precision and effectiveness. Developing tolerant genotypes with high productivity is undoubtedly the best environment friendly solution under such situations, but is not the easiest one. Progressesin understanding genetics of abiotic stress tolerance reveal that each stress response is controlled by multiple complex biosynthetic pathways with some key enzymes, proteins or secondary metabolites regulating the stress response. Moreover, many such response or tolerance expressions are controlled by group of genes or quantitative trait loci (QTLs). Identification of such QTLs in a population and its utilization in developing new improved genotypes depend on successful genotyping and precise phenotyping (Salekdeh et al., 2009). In the current age of genomics, genome sequences of many crops have been decoded and their transcriptomics under stress conditions have identified several new candidate genes and QTLs for abiotic stress tolerance. Two approaches are well established for utilizing these genomic resources for crop improvement. The first approach is to transfer the target gene/QTL through hybridization and identification of better performing segregants utilizing marker assisted selection (MAS) methods, which is limited to primary or secondary genepool of the donor species. The resultant success of marker assisted QTL introgression has been very effective in transferring waterlogging stress tolerance in rice. The Swarna Sub1 variety which has been developed by marker assisted transfer of *sub1*gene, is a real success in the flooded areas of Eastern India where it has been observed to withstand more than 10 days of waterlogging (CGIAR, 2012). Such technologies, once developed after long search, would have high impact in combating abiotic stress.

The second one is transfer of key gene(s) through transgenic technology for improving stress response, which is not limited within genepool, but is challenged by the difficulties in methodology, biosafety processes involved and social acceptanceof transgenic crops. The difficulties in developing transgenic plants for abiotic stress tolerance involving multiple genes are much more than targeting single gene based change in genetic make-up. Moreover, current understanding of tolerance to some of the abiotic stresses such as drought is not well defined at cellular level, thus devising a simple transgenic system may not be effective or easy. The time period for developing a transgenic plant is at least 3-6 years for research and development and a typical dormancy period of 6-10 years before reaching farmers field. Serious concerns have been placed whether under rapid climate change scenario a transgenic developed for a specific purpose may be effective after 9 (rapid release) - 16 (delayedrelease) years when climate might have changed. Given the heavy investments required for developing transgenic crops, such issues have been found to be crucial in determiningtarget traits andpolicies for transgenic research. However, the benefit of transgenic research is not only developing direct end product or varieties, but also to develop effective donor genotypes for crop breeding programmes. The transgenic lines can be integrated and used in many breeding programmes asdonor genotypes, which has been most successful in developing many BT-transgeic varieties of maize and cotton.

Whatever may be the approach, precise and accurate phenotyping is an integral component in identifying the stress response at every step from identification of tolerant genotype to development of the end product genotype with better yield and stress tolerance. Given the multiple facets of each stress response mechanism, clear-cut phenotyping or phenomics of stress tolerance is the need of the day. Phenomics is defined as acquisition of phenotypic data of an organism for a particular trait or a group of traits (Haule et al., 2010). It involves analysis of the phenotype at different levels (from macro level trait expression (group of traits expressed together in response to environment-individual trait expression-expression at tissue/ organ level-expression at cellular level) linked to the genotypic expression. The phenomics analysis considers two major phenotype platforms, one determined by the genotypic framework of the organism (internal phenotype) and the second one is the final expression of the internal phenotype at different environments (external phenotype).

The task of phenomics becomes more complicated for the abiotic stress, since the test environment is poorly defined. For example, the critical soil moisture causing drought will not affect every crop in similar fashion. Even for a single crop, drought may appear in different time period depending on the environment, such as at germination, early growth, pre-flowering or post-reproductive phases. Moreover, the change in soil moisture under drought largely depends on the soil structure and texture. A deep root system may be useful for combating drought where soil moisture is retained at lower soil layers, but it will not be very useful if the soil is sandy loam, where moisture is poorly retained. Similarly, osmotic adjustment, which plays a major role in drought tolerance in cereals, seems to be of less importance in drought tolerance mechanism of legumes. Thus defining a good phenotype for abiotic stress tolerance is dependent on many factors including soil, climate, and cropsystems (Figure 1). We will discuss some of the traits suitable for phenotyping in crop plants primarily taking drought stress as an example, since drought is considered to be the most threatening globally distributed abiotic stress to agriculture.

2. Morphomeric and Physio-chemical Traits for Phenomics of Stress Response

Root volume, root length and root/shoot ratio are considered to be important root traits for screening drought stress. The root-shoot ratio in almost all the crop plants increases under drought stress, indicating root growth is a common adaptation across species under water limited environments. A deep root system is desirable for imparting drought tolerance in crop plants. In most of the landraces of crop plants adapted to arid region, deep root system has been observed to be major factor contributing to drought tolerance (Trethowan and Mujeeb-Kazi, 2008).

Under drought condition, root hydraulic conductivity also plays key roles for adaptation to stress. For example, hydraulic conductivity of rice root is lower than maize root due to barriers created by endodermis and aerenchyma (Miyamoto et al., 2001). During water shortage, cavitations in water transport channel are created resulting in loss of connection between root and shoot, making rice less adapted to drought prone environments than maize.

A simple technique has been developed by Maiti (2012b) to screen drought tolerance in crop plants. It consists of exposing crop cultivars to different drought cycles depending on environments and crop species. Robust and deep roots are the characteristics of drought tolerant crop cultivars such as in cotton, maize (Maiti, 2012c), Maiti (2012d) developed low cost technology for improving crop productivity under various abiotic stresses. With respect to salinity he developed a simple semi-hydroponic technique for screening and selecting crop genotypes for salinity tolerance. Profuse roots located in the upper soil layers contributes osmotic adjustment of salt tolerant genotypes. Emergence percentage, root elongation, and production of profuse lateral roots are indicators for salinity tolerance (Maiti et al., 2012).

Drought condition during vegetative stages reduces growth and development of vegetative tissues and induces cellular death by disturbing osmotic potential. Grasses roll their leaves in response to drought stress, which is a common phenotypic signal of drought stress in the field. Drought tolerant genotypes exhibit delayed or less leaf rolling than the drought sensitive genotypes, hence it is a very important trait for selection against

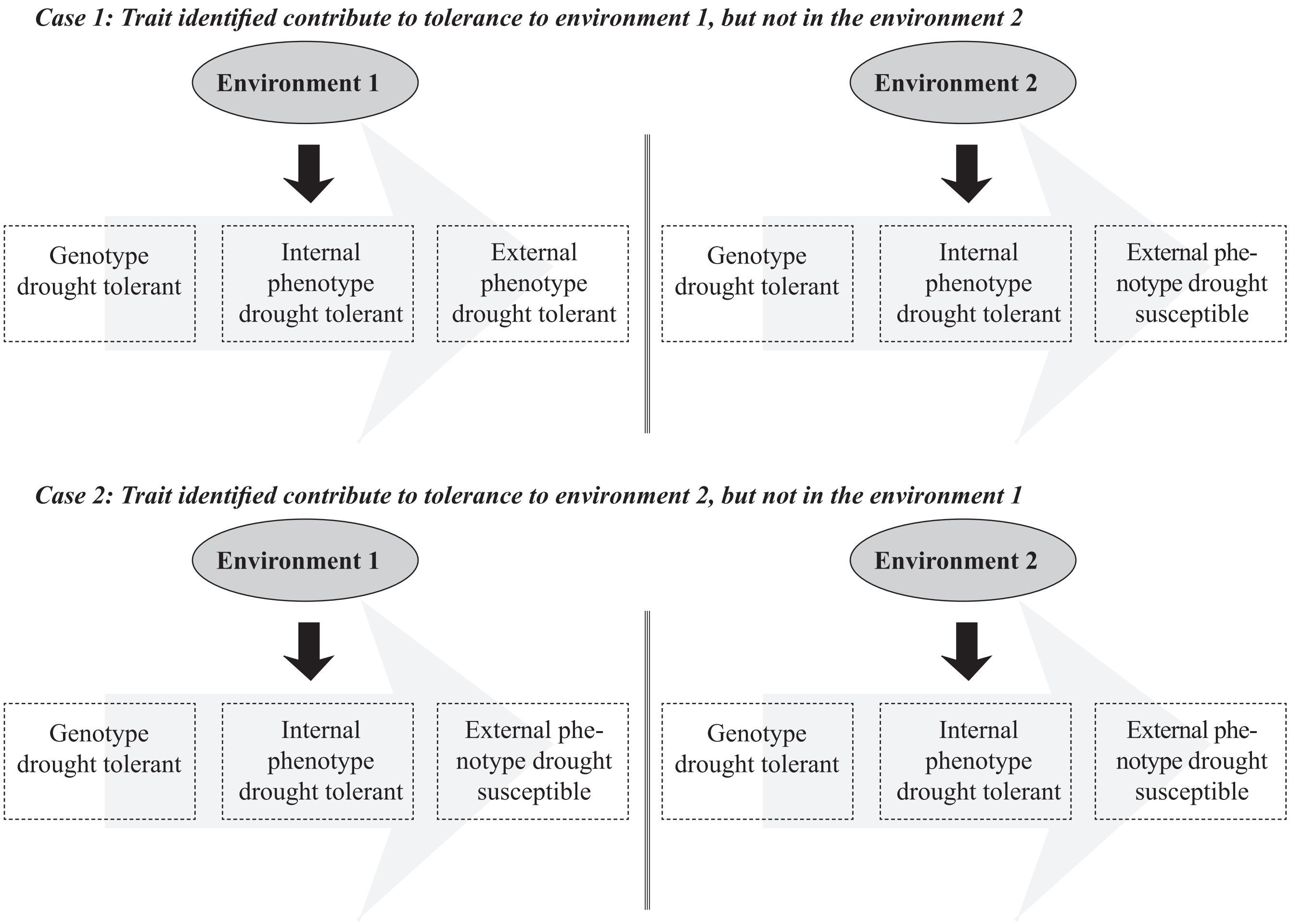

Figure 1: Crop tolerance to environment

drought stress in breeding programmes.

If onset of drought coincides with pre-flowering or flowering stage, fertilization is hampered as a result of which seed development is impaired. Under water stress, remobilization of reserves help in the rapid grain filling, poor filling leads to chaffy grains. For example, chaffy grain is a typical indicator of pre-flowering water stress in rice. The stay green trait in cereals, which results from delayed senescence of leaves due to slower rate of chlorophyll breakdown, is also considered an important character for drought tolerance. This phenotype contributes to better grain filling in cereals during post anthesis period, since the greener leaves produce more photosynthates during this period and translocate it to the endosperm of developing seeds.

3. Anatomical Traits Suitable for Phenomics of Stress Response

Advances in microscopy research and image processing technologies have opened up new possibilities for utilizing morpho-anatomical features for screening genotypes exhibiting abiotic stress tolerance. Utilizing such feature many new phenomic analysis protocols have been developed in recent past. For example, non-destructive imaging of plantsenescence process hasenabled researchers to measure drought or salt stress-induced changes in plant growth and anatomy over time.

The major anatomical features contributing to drought tolerance attempts to maximization of water conservation by modifying stomatal conductance and cuticular structures, maintenance of photosynthetic activities, economization of water distribution, efficient mobilization of storage reserve and enhanced water uptake through development of deep primary root system. Many drought tolerance genotypes have developed special structural mechanisms for maintaining the water requirement. One of the major objectives of such avoidance mechanisms is to maintain the leaf water potential by reducing transpiration. For example, stomatal activity is a primary indicator of drought stress. The stomata can sense drought condition through increase in abscisic acid (ABA) concentration, which cause efflux of solute from guard cells resulting in closure of stomata. It can sense rise in ABA concentration in nearer leaf tissues as well as in distant regions such as roots through xylem mediated ABA transport. Rapid stomatal sensitivity under drought stress is a desirable mechanism to close the stomata and reduce water loss (Maiti et al., 2012). Moreover, the ability to conserve water under drought stress depends on the thickness and deposition of cuticle.

The cortex and xylem cell wall of root thickens in response to drought stress, resulting in reduction in diameter of xylem vessels which limits water movement. Common bean cultivars that are susceptible to drought exhibit reduction in thickness of epidermis, protoderm and area of parenchymatic cells along with plasmolysis and death of root cells (Pena-Valdivia et al., 2010).

4. Biochemical Traits for Phenomics of Stress Response

Increased rootgrowth under drought condition is activated by and associated with a number of signalling processes, including ABA accumulation, concentration of reserve carbohydrates in the apical zone of root, accumulation of proline, cytokinins and expansion proteins. Proline provides tolerance to abiotic stress via osmoprotection as well as by increasing the antioxidant enzyme activities.The soluble sugars also contribute to stress tolerance as signaling agents, osmoprotectans and pool for metabolic activities. Studies have shown that accumulation of these metabolites is much higher in drought tolerant cultivars of rice, wheat and sorghum than the susceptible cultivars. ABA, sugars like trehalose and ion channels are key indicators of most of the abiotic stresses, although their responses are not similar under every stress conditions or every plant parts. For example, the drought tolerant rice cultivars adapt to the water limited environment by accumulating more soluble sugars and proline for higher osmoprotection in the leaf sheaths than leaf blades. Lipid peroxidation is considered as another key indicator of drought stress. Plants under drought stress exhibit increased lipid peroxidation activity, which is associated with susceptibility to drought stress (Pandey et al., 2010). On the other hand, higher activity of antioxidant enzymes like peroxidase, catalase and superoxide dismutase are associated with better tolerance to drought stress.

5. Conclusion

Comprehensive phenomics of abiotic stress response thus need to be characterized by integrating trait information from various phenotypic interfaces to fit the phenomic data in the genotypic array and draw a meaningful conclusion on response to abiotic stresses, particularly to identify key genes for their further use in crop breeding programmes and for manipulating stress response by further up and down regulationof the target genes.

6. References

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